#10 7/22/00 Declaration

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date appearing below.

ELI LILLY AND COMPANY

By Cynthia Craft Date July 7, 2000

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Hermeling, Ronald N., *et al.*	)
Serial No.	:	09/209,799	) Group Art Unit: 1653
Filed	:	December 11, 1998	)
For	:	GLUCAGON-LIKE PEPTIDE-1 CRYSTALS	) Examiner: F. Moezie
Docket No.	:	X-10242	)

DECLARATION UNDER 37 CFR 1.132 OF CHAKRAVARTHY NARASIMHAN, PH.D.

Assistant Commissioner for Patents
Washington, D.C. 20231
Sir:

I, Chakravarthy Narasimhan of 10744 Putnam Place, Carmel, Indiana, 46032 hereby state and declare that:

1. I am an inventor of the above referenced U.S. Patent Application Serial No. 09/209,799 (hereinafter the "'799 Application"). I have read and thoroughly understand the '799 Application. I have also read and understand the latest Office Action for this application, which was mailed from the Patent Office on January 7, 2000, as well as the references cited in the Office Action. These references include EP 619,322 by Danley *et al.*, U.S. Patent No. 5,977,071

to Galloway *et al.* and Kim *et al., Pharmaceutical Research 12:*1664 (1995) (hereinafter "Kim *et al.*").

2. The '799 Application is directed to a composition comprising individual flat rod shaped or plate-like crystals of the glucagon-like peptide-1 compounds (hereinafter "GLP-1 compounds") and to methods of preparing said composition. Valine-8-glucagon-like peptide-1 (hereinafter " Valine-8-GLP-1(7-37)OH") is one example of a GLP-1 compound.

3. GLP-1 compounds are biologically active polypeptides that have been shown to control blood glucose levels in Type II diabetic patients. Administering the zinc-containing crystalline form of GLP-1 compounds has the advantage of resulting in extended time action compared with administering GLP-1 compounds orally or by injecting a solution of GLP-1 compounds. The crystalline form of GLP-1 compounds is administered by suspending crystals of the compound in a suitable liquid carrier to form a crystal suspension. The crystal suspension is then shaken gently so that the crystals are suspended uniformly and homogeneously throughout the liquid medium. The appropriate dose is then drawn into a syringe and injected.

4. To ensure that uniform and correct doses are reproducibly administered to the patients for each injection, it is critical that the crystals remain homogenously and uniformly suspended throughout the liquid medium while the crystal suspension is being drawn into the syringe. Thus, crystals which remain

suspended in the carrier medium for longer periods of time are preferred over crystals which remain suspended for shorter periods of time.

5. The ability of a crystal to remain suspended in a liquid carrier, i.e., its suspendibility, can be assessed by the sedimentation rate, which is the final volume occupied by the crystals in a liquid medium after they have completely settled, divided by the time required to reach this final volume after having been resuspended. The suspendibility of the crystals is inversely proportional to this ratio. Thus, the suspendibility, i.e., the length of time which crystals remain suspended in solution, becomes greater as the sedimentation rate becomes smaller.

6. Experiments were performed to compare the suspendibility of the rod shaped and plate-like valine-8-GLP-1(7-37)OH crystals described and claimed in the '799 application with GLP-1 crystals prepared by methods disclosed in the prior art. These experiments were carried out by me or by others whose work I have thoroughly reviewed. These experiments show that the valine-8-GLP-1(7-37)OH crystals of the '799 application have greater suspendibility than valine-8-GLP-1(7-37)OH crystals prepared by crystallization methods described in EP 619,322 and in Kim *et al*. These experiments and the results thereof are described in Sections 7-8 of this Declaration.

7. The following procedures were used to prepare crystals of valine-8-GLP-1(7-37)OH:

   a. Procedures described in Example 36 of EP 619322 were followed. Briefly, valine-8-GLP-1(7-37)OH (9.79 mg/mL) was crystallized from an aqueous solution containing 22.5% polyethylene glycol at pH 8.8. Clusters of microcrystals and clusters of needles were obtained. Individual crystals were not obtained.

   b. Procedures described in Example 45 of EP 619322 (4.43 mg/mL) were followed. Briefly, valine-8-GLP-1(7-37)OH was crystallized by dissolving in an aqueous solution containing 1% sodium sulfate at pH 7.87 and then adjusting the pH to 6.1. Obtained were clusters of plates and clusters of needles. Individual crystals were not obtained.

   c. Procedures described in the first full paragraph on page 1665 of Kim *et al.* were followed. Briefly, valine-8-GLP-1 (7-37)OH (4.2 mg/mL) was crystallized by dissolving in an aqueous solution containing 1% sodium chloride at pH 9.5 and then adjusting the pH to 6.7. Obtained were clusters of plate-like crystals. Only one individual needle-like crystal was observed.

   d. The procedure described in Example 17 of the subject application was followed. Briefly, valine-8-GLP-1(7-37)OH (2.5 mg/ml) was crystallized from an aqueous solution containing 5% ethanol and 1.0% ammonium sulfate at pH 6.4. Obtained were clusters of plate-like crystals and individual plate-like crystals.

The sedimentation rate of each sample of crystals produced in subsections a-d above was determined and is shown in The Table below:

The Table

Sample	Sedimentation Rate
Example 36 from EP 619322	0.04 ml/minute
Example 45 from EP 619322	0.13 ml/minute
Procedure from the Kim *et al.*	0.03 ml/minute
Procedure from Example 17 of the subject application	0.01 ml/minute

8. From the data presented in The Table in Section 7, it can be seen that the plate-like crystals obtained using the procedure described in Section 7d (Example 17 of the subject application) have the lowest sedimentation rate of all samples tested. Thus, these crystals remain suspended the longest of all of the crystals tested and are therefore preferred when administered by injection as a liquid crystalline suspension.

9. The prior art crystallization procedures described in Sections 7a-7c of this Declaration utilize aqueous crystallization solutions or aqueous crystallization solutions with polyethylene glycol. The method described in Section 7d above differs from the prior art methods in that ethanol is present in the crystallization solution. It appears to be the presence of ethanol which results in the superior sedimentation properties of the valine-8-GLP-1(7-37)OH crystals prepared by the method described in Section 7d of this Declaration (Example 17 of the subject application).

10. U.S. Patent No 5,977,071 describes and claims crystalline GLP compounds (see column 10, lines 26-28 and Claims 27-34). However, U.S. Patent No 5,977,071 provides only one crystallization procedure, which is described in Example 2 (column 12) for GLP-1(7-36)NH_2. In Example 2, GLP-1(7-36)NH_2 is crystallized from HEPES buffer at pH 7.4 containing zinc. HEPES buffer is aqueous and contains no ethanol. As noted in Section 9 of this Declaration, ethanol in the crystallization solution appears to be required to produce crystals having superior sedimentation properties.

11. Experiments were also performed in which attempts were made to crystallize valine-8-GLP-1(7-37)OH in the presence of *m*-cresol. Crystalline material was not obtained. This result is consistent with results reported in the paragraph bridging the left and right columns on page 1666 of Kim *et al.*, which states that crystals of GLP-1(7-37)OH became amorphous after contacting *m*-cresol. These results and the results reported in Kim *et al.* lead me to conclude that crystalline material is either not obtained when following the procedure in Example 44 of EP 619,322 or does not have the desirable sedimentation properties of those prepared by the method described in Section 7d of this Declaration. Example 44 of EP 619,322 describes formation of crystalline GLP-1(7-37)OH from a slurry containing ethanol, phosphate buffered saline and *m*-cresol. Example 44 uses nine times as much ethanol as the slurry. In contrast, the crystallization method disclosed in the '799

Application generally uses between about 2-15% ethanol in the crystallization solvent.

12. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Chakravarthy Narasimhan, Ph.D.

7/7/00
Date